PATENT APPLICATION

Method and Apparatus for Inspecting DNA and Method for Detecting Fluorescence

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METHOD AND APPARATUS FOR INSPECTING DNA AND METHOD
FOR DETECTING FLUORESCENCE

BACKGROUND OF THE INVENTION

The present invention relates to a method and an apparatus for irradiating DNA marked with fluorescence or a fluorescent material with excitation light

5 to inspect the DNA and a fluorescence detection method and more particularly to a method and an apparatus for detecting and inspecting DNA marked with a plurality of kinds of fluorescence samples or a plurality of kinds of fluorescent materials at high speed.

10 As a method for irradiating fluorescence-marked DNA with excitation light to inspect the DNA, there is a method for focusing laser light constituting excitation light on a sample as a single spot beam to detect fluorescence and collecting a fluorescence image

15 by means of scanning of the excitation light and the sample. In detection of a plurality of fluorescent marks, a desired all area of a sample is first scanned with first excitation light to obtain a fluorescent image of a first fluorescent mark and the desired all

20 area of the sample is then scanned with second excitation light again to collect the fluorescent image of a second fluorescent mark.

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SUMMARY OF THE INVENTION

It is an object of the present invention to provide a high-speed detectable DNA inspection method and apparatus.

5 In the conventional method, a desired area is scanned with excitation light having different wavelength in accordance with a plurality of fluorescent marks and this scanning is repeated by the number of marks. In order to change a relative position of a

10 single spot beam and a sample to inspect a desired area at high speed, it is necessary to move a stage on which the sample is placed at high speed. However, when the stage is moved at high speed, a considerable time is required for acceleration and deceleration for driving

15 the stage since reciprocating motion is needed.

Further, when the scanning using the stage is repeatedly made for each wavelength of the excitation light, a positional shift or deviation occurs in a combined image of fluorescent images obtained by means

20 of excitation lights when a driving accuracy of the stage and a reproduction accuracy are not sufficient. Consequently, high-speed and high-accuracy inspection of DNA required increasingly in future cannot be attained.

25 In order to solve the above problems, the present invention comprises measures described below.

A sample having a DNA piece added with a plurality of L kinds of fluorescence-marked materials

combined to corresponding DNA is irradiated with a
plurality of M kinds of minute spot excitation lights
in accordance with the fluorescence-marked materials.
Fluorescence intensities obtained in accordance with
5 the fluorescence-marked materials are separately
detected by means of the plurality of M kinds of minute
spot excitation lights. The separately detecting
operation of the plurality of fluorescent marks is made
by changing a position on the sample irradiated with
10 the spot excitation lights over a desired area by the
number of times smaller than the number L of kinds of
the fluorescence-marked materials, more preferably
once, in order to detect the fluorescence intensities,
to thereby inspect the DNA added with the plurality of
15 kinds of fluorescence-marked materials.

Further, when the plurality of kinds of
minute spot excitation lights are a plurality of minute
multi-spot excitation lights, respectively, a plurality
of points about the fluorescent marks can be detected
20 at the same time to attain high-speed detection.

When different positions on the sample from
one another are irradiated with the plurality of kinds
of multi-spot excitation lights, fluorescence
components from the fluorescent marks excited by the
25 plurality of kinds of excitation lights can be
separately detected with high accuracy without causing
fluorescence components to impede one another as noise.

Irradiation with the plurality of kinds of

multi-spot excitation lights is made at the same time

and fluorescence obtained by the plurality of kinds of

minute spot excitation lights in accordance with the

fluorescence-marked materials is detected by a

5 plurality of weak-light detection elements in accord-

ance with respective excitation lights. The fluores-

cence intensities obtained in accordance with the

fluorescence-marked materials are detected separately.

Thus, in the photon-counting method used particularly

10 when weak fluorescence is detected, the photon-counting

can be made at the same time with respect to respective

fluorescence components to make inspection at high

speed in wide dynamic range.

Irradiation with the plurality of kinds of

15 minute spot excitation lights is made in time series

manner in accordance with wavelengths of the excitation

lights and fluorescence obtained by the plurality of

kinds of minute spot excitation lights in accordance

with the fluorescence-marked materials is detected by a

20 common weak-light detection element to respective

excitation lights. The fluorescence intensities

obtained in accordance with the fluorescence-marked

materials are detected separately. Thus, in detection

using the multi-spot, particularly, the weak-light

25 detection element and its peripheral circuit become

inexpensive.

Substantially the same position is irradiated

with the plurality of kinds of minute spot excitation

lights. Thus, detection by means of respective excita-
tion lights can be made at the same place and detection
with high accuracy can be made without any shift or
deviation due to yawing and rolling of a stage in a
5   combined image.

The plurality of kinds of minute spot excita-
tion lights are turned on and off in different time
zone within a time that a relative position of the spot
excitation lights and the sample to be irradiated is
10   changed by substantially one pixel. Thus, the respec-
tive fluorescent marks can be detected with high
accuracy without mixing noise one another.

The plurality of kinds of minute spot excita-
tion lights are changed stepwise at respective excita-
15   tion light intensity levels within a time that a
relative position of the spot excitation light and the
sample to be irradiated is changed by substantially one
pixel to detect the fluorescence intensity at each
step, so that detection is made over a wide dynamic
20   range at a high speed.

The DNA inspection apparatus according to the
present invention is configured as follows. That is,
the DNA inspection apparatus comprises one to a
plurality of light sources for emitting lights having
25   wavelengths different from one another, a plurality-of-
wavelength excitation optical system for irradiating a
DNA sample added with a plurality of fluorescence-
marked materials with lights having the plurality of

wavelengths from the light sources as minute spot excitation lights, a wavelength separation fluorescence detection system for separately detecting fluorescence intensities obtained by the respective excitation

5   lights in accordance with the fluorescence-marked materials, a driving stage for changing a relative position of the minute spot excitation lights and the DNA sample over a desired area, and a processing unit for driving the driving stage so that the relative

10 position of the minute spot excitation lights and the DNA sample is changed over the desired area by the number of times smaller than the number of kinds of the fluorescence-marked materials so as to construct image information of the plurality of fluorescence-marked

15 DNAs on the sample from fluorescence detection informa-tion obtained by the detection system and the stage position information obtained by scanning by the number of times smaller than the number of kinds of the fluorescence-marked materials over the desired area.

20   Further, the present invention is applied to not only inspection of the fluorescence-marked DNA but also general fluorescent material emitting fluorescence peculiar to molecules such as protein.

        According to the present invention, DNA to be

25 inspected including the plurality of fluorescent marks or the samples containing the plurality of kinds of fluorescence materials cab be detected by scanning within a desired detection spread by the number of

times smaller than the number of kinds of the samples

containing the fluorescence materials or fluorescent

marks, more preferably once, to thereby make it

possible to attain detection extraordinarily fast as

5   compared with the prior art. Consequently, a large

number of objects to be inspected can be detected and

inspected at high speed to attain largely temporal and

economical effects.

BRIEF DESCRIPTION OF THE DRAWINGS

10   Fig. 1 is a perspective view illustrating the

whole structure of a DNA inspection apparatus according

to the present invention;

        Fig. 2 is a plan view showing multi-spot

excitation light within a detection field of vision

15   according to the present invention;

        Fig. 3 is an enlarged plan view of multi-spot

excitation light according to the present invention;

        Fig. 4 is a front view showing part of an

optical system in which paths of excitation light and

20   fluorescence according to the present invention are

shown;

        Fig. 5 is a front view showing an example of

a fluorescence detection optical system according to

the present invention;

25   Fig. 6 is a front view illustrating a photo-

multiplier showing an example of fluorescence detection

spots on the photo-multiplier according to the present

invention;

Fig. 7 is a front view illustrating a photo-multiplier showing another example of fluorescence detection spots on the photo-multiplier according to the present invention;

Fig. 8 is a diagram representing a relation of a plurality of fluorescence samples and the photo-multiplier according to the present invention;

Fig. 9 is a perspective view showing a plurality-of-wavelength excitation optical system according to the present invention;

Fig. 10 is a graph showing a relation of temporal change in excitation light intensities and detection timings according to the present invention;

Fig. 11 is a graph showing a relation of temporal change in excitation light intensities and detection timings according to the present invention;

Fig. 12 is a graph showing a photon counting and detecting method according to the present invention; and

Fig. 13 is a perspective view showing a fluorescence detection unit according to the present invention.

DESCRIPTION OF THE EMBODIMENTS

Fig. 1 illustrates an embodiment according to the present invention. Numeral 1 denotes a control unit and numeral 5 denotes a sample board on which a

sample including fluorescent marks or fluorescence

substance is placed. Numerals 21 and 22 denotes an

excitation light source and a plurality-of-wavelength

excitation optical system for irradiating the sample

5   with minute spots of excitation light, respectively,

described later with reference to Fig. 9. That is, the

excitation light source 21 constitutes part of a light

source composed of a plurality of semiconductor lasers

having a wavelength of, for example, 635 nm and an

10   excitation optical system for irradiating the sample

with minute multiple spots of light emitted from the

light source. The plurality-of-wavelength excitation

system 22 constitutes part of a light source formed of

a YAG laser having a wavelength of 532 nm and an

15   excitation optical system for irradiating the sample

with minute multiple spots of laser light emitted from

the light source. The number of minute multiple spots

is 64, 32, 16 or 8 and the number of minute multiple

spots is selected therefrom in accordance with a

20   specification of an inspection system. 64 laser beams

having the wavelengths of 635 nm and 532 nm emitted

from the excitation light source 21 and the plurality-

of-wavelength excitation system 22, respectively, form

64 multi-spot spatial images having the respective

25   wavelengths on positions 21A and 22A by means of beam

alignment units 2101 and 2201, respectively. The

multiple spot spatial images are focused on the sample

put on the sample board 5 as multi-spot excitation

images indicated by 21B and 22B as shown in Fig. 2 by means of a tubular lens or a focusing lens 24 and an objective lens 3.

More particularly, as shown in Fig. 3, the M
5 (64) spots each having a diameter d are arranged at a pitch Py on the sample. Numeral 21B represents the multi-spot having the wavelength of 635 nm and numeral 22B represents the multi-spot having the wavelength of 532 nm. The spaces Px between the spots 21B and 22B
10 are not equal to one another and are several tens to several hundreds micrometers in the embodiment. The spaces can be made equal to one another depending on a specification to be targeted as described later.

Numerals 31 and 32 of Fig. 1 denote wave-
15 length selection beam splitters which transmit light beams having wavelengths of 635 and 532 nm of the excitation light, respectively. When fluorescence material on the sample is excited by the multiple spots, the fluorescence material absorbs the excitation
20 light having the wavelength of 635 nm ($\lambda 1$) impinging on positions 2101, 2102, ..., 21M of the excited minute spots and emits fluorescence having a wavelength $\lambda 1'$ slightly longer than the above wavelength when fluores-cence material ML1 emitting fluorescence is present.
25 The emitted fluorescence passes through the objective lens 3 having a large numerical aperture (NA) as shown in Fig. 4 and advances as shown by broken line with double arrows. The emitted fluorescence is then

reflected by the wavelength selection beam splitter 32
and further reflected by wavelength selection beam
splitters 33 and 34 having the same wavelength
selection characteristic as the beam splitter 32 (or

5   reflected by a mirror 33' having a high reflectivity
and a wavelength selection beam splitter 34' which
transmits fluorescence $\lambda 2'$ and reflects $\lambda 1'$ as described
later) to enter a wavelength separation fluorescence
detection system 100 described later in detail with

10   reference to Figs. 5 to 8. The wavelength selection
beam splitters 33 and 34 having the wavelength
selection characteristic reflect the fluorescence to
thereby cut excitation lights $\lambda 1$ and $\lambda 2$ considerably.

On the other hand, positions 2201, 2202, ...,

15   22M on the sample are irradiated with the multi-spot
excitation light having the wavelength of 532 nm ($\lambda 2$)
different from the wavelength $\lambda 1$. The sample absorbs
the multi-spot excitation light and when fluorescence
material ML2 emitting fluorescence is present, fluores-

20   cence having the wavelength $\lambda 2'$ slightly longer than
the above wavelength is produced. The emitted fluores-
cence also shown in Fig. 4 passes through the objective
lens 3 having the large numerical aperture (NA) and
advances as shown by broken line with double arrows.

25   The fluorescence then transmits the wavelength
selection beam splitter 32 and is reflected by wave-
length selection beam splitters 31. This reflected
fluorescence transmits the wavelength selection beam

splitter 34 (or 34') to enter the wavelength separation fluorescence detection system 100. When the fluorescence is reflected by the wavelength selection beam splitter 31 and transmits the wavelength selection beam

5   splitter 34 or 34', the excitation lights $\lambda 1$ and $\lambda 2$ are cut considerably.

The relative position of the multi-spot excitation lights having a plurality of wavelengths and the sample is scanned once and a plurality of fluores-

10   cent marks or information of the fluorescence material detected by the wavelength separation detection system of Fig. 1 is stored once. When desired inspection specifications such as, for example, a coordinate range of an inspection area, information relative to an

15   object to be inspected and output conditions are inputted by an input terminal 13 of Fig. 1, detection is made in accordance with the conditions and a desired output of detection results obtained is displayed on a display monitor 12, for example. Further, the results

20   can be transmitted to a distant place through a line 14. Inputting of conditions of the above apparatus, control of the excitation light, the stage and detection of a photo-multiplier, processing of the result, outputting of the result and the like are controlled by

25   the control unit 1 of Fig. 1.

Fig. 5 shows an embodiment of the wavelength separation fluorescence detection system 100. Numeral 1001 denotes a relay lens or a focusing lens. The

fluorescence components emitted from different fluores-
cence materials ML1 and ML2 pass through the lens 1001
and are then separated into respective light fluxes at
positions of interference filters 1211 and 1221. This
5   separation is realized by shifting a position on the
sample irradiated with both the excitation lights.
Further, the separation is also realized by adjustment
of positions or angles of the wavelength selection beam
splitters 33 and 34. The separated fluorescence
10   components $\lambda 1$ and $\lambda 2$ pass through the interference
filters 1211 and 1221 to thereby reduce the excitation
light component to an extremely small amount and remove
noise in detection of fluorescence.

The fluorescence components $\lambda 1'$ and $\lambda 2'$
15   passing through the interference filters are reflected
by wavelength selection mirrors 1212 and 1222 (or high-
reflecting mirrors 1212' and 1222' of broadband) and
enter a multi-channel photo-multiplier 101 shown in
Fig. 6. Since the wavelength selection mirrors 1212
20   and 1222 transmit the excitation light component and
reflect the fluorescence, the excitation light
component is reduced to an extremely small amount equal
to almost 0 after the reflection of the fluorescence.
Since the wavelength selection mirrors 1212 and 1222
25   have respective reflecting planes which are not formed
on one plane as shown in Fig. 5 and are inclined at an
angle, both the fluorescence components reflected by
the wavelength selection mirrors are focused on

pinholes of a pinhole array or slits formed in a line at the front of the multi-channel photo-multiplier 101 as shown in Fig. 6.

Fig. 7 shows an embodiment showing a detec-
5 tion method effective for the case where the multi-channel photo-multiplier 101 is long in a direction perpendicular to the array direction. In this case, the mirror plane of the wavelength selection mirrors 1212 and 1222 of Fig. 5 is disposed on one plane.
10 Further, when the excitation light can be cut suffi-ciently until this mirror is reached, a single usual mirror may be used.

In the embodiment, since a plurality of fluorescence components are detected by the single
15 photo-multiplier (multi-channel photo-multiplier), the excitation light is turned on and off at different time zones. Semiconductor lasers having a wavelength of 635 nm and constituting the excitation light source are turned on and off by turning on and off a signal for a
20 power supply for driving the lasers. On the other hand, a beam of the YAG laser in the plurality-of-wavelength excitation system 22 is turned on and off by means of an acousto-optic modulator (AO modulator) not shown.

25 Intensities I21 and I22 of the excitation lights having the wavelengths of 635 nm and 532 nm, respectively, have time zones different from each other as shown by (A1) and (B1) of Fig. 10 and in which the

excitation lights are turned on and off. The turning
on time of the excitation lights is several tens μs to
several hundreds μs. In almost all fluorescence
materials, since a delay time in emission of fluores-

5   cence after excitation is several ns to several
hundreds ns, fluorescence is emitted in almost concur-
rence with an irradiation time of the excitation light.
Accordingly, respective fluorescence components are
detected in synchronism with turning on and off of the

10 excitation light as shown by (A2) and (B2) of Fig. 10.
(C) of Fig. 10 shows a relation of a movement amount or
positional information St of the stage for holding the
sample in synchronism with turning on and off of the
excitation light and detecting fluorescence at

15 different positions and time. That is, movement is
made by Δx during a time Δt for detecting fluorescence
components at 64 spots in response to turning on and
off of the two excitation lights. This movement
distance can be made approximately equal to a diameter

20 d of the spot to thereby detect fluorescence in a
desired area by means of scanning and turning on and
off.

Fig. 8 shows an embodiment according to the
present invention in which irradiation with excitation

25 lights having a plurality of wavelengths are made at
the same time to detect fluorescence at the same time.
In this case, DNA sample or fluorescence material
sample is irradiated with two (or three or more)

excitation lights concurrently at the same place thereof (a different place may be irradiated). When the same place is irradiated with the excitation lights having the plurality of wavelengths, positions and

5   inclinations of the wavelength selection beam splitters 31 and 34 are previously adjusted. With such adjustment, fluorescence emitted by the excitation light having the wavelength of 635 nm, for example, can pass through the interference filter 1211 of Fig. 8 and

10   fluorescence emitted by the excitation light having the wavelength of 532 nm can pass through the interference filter 1221 of Fig. 8. Consequently, the respective fluorescence components can be reflected by the mirrors 1212' and 1222' and enter different multi-channel

15   photo-multipliers 1011 and 1012 to thereby detect the fluorescence components separately at the same time.

        Fig. 9 is a diagram showing an embodiment according to the present invention in which three kinds of excitation lights are used by way of example.

20   Numeral 210 denotes a multi-spot excitation light system using a semiconductor laser having a wavelength of 635 nm ($\lambda 11$), 220 a multi-spot excitation light system using a wavelength of 590 to 600 nm ($\lambda 12$), and 230 a multi-spot excitation light system using a laser

25   having a wavelength of 532 nm ($\lambda 13$). A method of converting lights emitted from the respective light sources into multi-spots will be described using the multi-spot excitation light system 210.

The beam emitted from the light source is shaped to have a desired beam diameter and spread angle by means of a beam shaping unit not show in drawings and is converted to have a desired polarization state

5 by a polarizer 212. The polarized beam passes through beam splitters 213, 214 and 215. The beam splitters are each composed of an isosceles right triangle and a parallelogram with apical angles of 45 degrees having a hypotenuse stuck on a hypotenuse of the isosceles right

10 angle and the stuck surface or side constitutes a beam splitting surface. Further, when the distance between the stuck side of the parallelogram and its opposite side of the beam splitter 213 is d, the corresponding distances of the beam splitters 214 and 215 are 2d and

15 4d, respectively. Consequently, the beam passing through the beam splitter 215 is increased to 8 beams and the space between the beams is equal to $\sqrt{2}d$. The 8 beams form multiple spots having the desired beam diameter on 21A to 21B lines 2100 by means of convex

20 lenses array 2161 to 2168.

Numerals 220 and 230 of Fig. 9 also represent multi-spot excitation optical systems having such structure. A wavelength selection beam splitter 202 transmits excitation light having a wavelength $\lambda 12$ and

25 reflects excitation light having a wavelength $\lambda 13$. A wavelength selection beam splitter 201 transmits excitation lights having wavelengths $\lambda 12$ and $\lambda 13$ and reflects excitation light having a wavelength $\lambda 11$.

The multiple beams having 8 beams of the excitation lights having three wavelengths are increased to 32 beams equal to four times by means of beam splitters 203 and 205 made of, for example,

5 calcite of birefringent material and wave plates 204 and 206 and form multiple spots by a desired beam system on straight lines 2200 and 2300 on surfaces 21A and 21B. The sample 5 is irradiated with the multiple spots of the three excitation lights formed on the

10 straight lines 2100, 2200 and 2300 in this space at a desired spot diameter by means of the tubular lens 24 and the objective lens 3 described in Fig. 1.

Fig. 11 shows an embodiment according to the present invention in which there are two excitation

15 wavelengths. In order to set a dynamic range for detection of fluorescence largely, it is necessary to change the intensity of excitation light to detect fluorescence. That is, when fluorescence having the number of molecules of fluorescence per unit area to be

20 detected equal to an extremely small value to a large value such as, for example, one molecule/μm^2 to 10 thousands molecules/μm^2 is to be detected, the detection resolution (detection range/noise level) of a high-sensitivity detector such as a photo-multiplier is

25 exceeded. Accordingly, this problem is solved by the method shown in Fig. 11. (A1) and (B1) of Fig. 11 have the abscissa representing time and the ordinate representing the intensities on the sample of

excitation lights having excitation wavelengths λ1 and λ2, respectively. In the embodiment, detection using the excitation lights λ1 and λ2 is made in the time series manner, while the following description can be similarly applied to the case where the detection is made at the same time. During time t0 to t2, excitation is made by the excitation light λ1 to detect fluorescence. During time t0 to t1, excitation is made by strong light I1A as the intensity I21' of excitation light and during time t1 to t2, excitation is made by weak light I1B as the intensity I21' of excitation light. As shown by (A2) and (B2) of Fig. 11, fluorescence is detected by the excitation light intensities I1A and I1B in the zones t0 to t1 and t1 to t2, respectively.

Similarly, during time t2 to t4, as shown by (B1) of Fig. 11, the excitation light intensity I22' for the excitation light of λ2 is set to I2A during time t2 to t3 and to I2B during time t3 to t4. As shown by (B2) of Fig. 11, respective fluorescence intensities are detected in respective time zones.

Fig. 12 shows a detection method of a signal of a photo-multiplier which can be applied to the case where the number of fluorescence molecules is very small to the case where it is large. When the number of fluorescence molecules is small, a photon-counting method is applied. The photon-counting method is effective for the case where light is weakened and the

number of photon pulses is about several counts to several hundreds or several thousands counts within a detection time. Conversely, when fluorescence is increased too much, photon-pulses are frequently

5 detected within a time width of a pulse signal and the pulses cannot be counted exactly. In such case, as described with reference to Fig. 11, by reducing the fluorescence light intensity extraordinarily to irradiate fluorescence with it, overlap of photon-

10 pulses can be reduced and fluorescence can be detected.

(A1) of Fig. 12 is similar to (A1) of Fig. 11 with the exception that the time axis of the abscissa is enlarged. In the embodiment, three kinds of fluorescence molecules are detected at the same time

15 while scanning the sample and the excitation light relatively. That is, the sample is irradiated with two or three kinds of excitation lights at the same time as shown by (A1) of Fig. 12. Relative values of the respective excitation lights are I1A, I2A and (I3A) and

20 I1B, I2B and (I1B) having extraordinarily weak inten- sity. Counts of photon-pulses of fluorescence obtained from three kinds of fluorescence materials L1, L2 and L3 are shown by (C1), (C2) and (C3) of Fig. 12. The fluorescence material L1 has approximately medium

25 intensity of fluorescence, L2 weak intensity, and L3 very strong intensity.

There are two pulse-counting methods includ- ing a first method of counting a pulse having even a

large pulse width as one pulse as shown in Fig. 12 and

a second method of measuring a time that a pulse signal

is high. In the first method, when the number of

pulses is increased, a count is reduced conversely, so

5   that the relation of the detection intensity and the

pulse count is not a single-valued function and the

count cannot be specified. On the contrary, in the

second method, even if the number of pulses is

increased, a count is not reduced and the relation of

10 the detection intensity and the pulse count is a

single-valued function, so that exact measurement can

be made by correcting the count. The second method can

make more exact counting, although when the width of

pulses is small, it is difficult to make exact counting

15 if the time constant of the counting circuit is not

made very small.

In the embodiment of Fig. 12, since irradia-

tion with strong excitation light and weak excitation

light is made to count photons in respective cases, the

20 counting can be made substantially without error even

in the first method that the single-valued function is

not obtained from the respective counts for the strong

and weak excitation lights. That is, in the case of

(C1) of Fig. 12, if a count obtained during time t0 to

25 t1 is not largely changed from a count obtained during

time t1 to t2, it is understood that the latter value

is correct. Further, as shown by (C3) of Fig. 12, when

a count obtained during time t0 to t1 is small and a

count obtained during time t1 to t2 is large, it is

understood that the latter value is correct. On the

other hand, as shown by (C2) of Fig. 12, when a count

obtained during time t1 to t2 is almost 0, it is

5 understood that the count obtained during time t0 to t1

is correct.

Moreover, when a wider dynamic range is

required, the intensity of the excitation light shown

by (A1) of Figs. 11 and 12 is set to three stages

10 including a very strong excitation light, an approxi-

mately medium excitation light, and a very weak

excitation light to thereby make it possible to attain

detection in a wider dynamic range.

Fig. 13 shows an embodiment according to the

15 present invention in which only a fluorescence detec-

tion portion is shown. In the excitation light

irradiation optical system, the wavelength selection

beam splitters 201 and 202 are aligned so that three

excitation lights 2100, 2200 and 2300 of Fig. 9 have

20 the same optical path. The sample 5 shown in Fig. 1 is

irradiated with three multi-spot excitation lights

2100', 2200' and 2300' at the same place so that

fluorescence components L1, L2 and L3 obtained are

detected as follows. The fluorescence component L1 is

25 reflected by the wavelength selection beam splitters 32

and 34 of Fig. 1 and the fluorescence components L2 and

L3 transmit the wavelength selection beam splitter 32,

are reflected by the wavelength selection beam splitter

31 and transmit the wavelength selection beam splitter
34 to thereby be led to the fluorescence detection
portion shown in Fig. 13. The fluorescence obtained
from the multi-spot excitation light on the sample is
5 focused on light receiving portion of the multi-channel
photo-multipliers 1011, 1012 and 1013 as a multi-spot
image by means of the objective lens 3 of Fig. 1 and
the tubular lens 1001 of Fig. 13. The fluorescence
components obtained from three fluorescent marks are
10 separated by means of wavelength separation beam
splitters 1231 and 1232 of Fig. 13. That is, the
fluorescence component L1 obtained by the excitation
light 2100' is reflected by the wavelength separation
beam splitter 1231, passes through an interference
15 filter 1241 which transmits only this fluorescence
component and then passes through an opening of a
multi-pinhole array 1010 to thereby be detected by the
multi-channel photo-multiplier 1011. Similarly, the
fluorescence components L2 and L3 pass through the
20 wavelength selection beam splitter 1231. The fluores-
cence component L2 transmits the wavelength selection
beam splitter 1232 and the fluorescence component L3 is
reflected by the wavelength selection beam splitter
1232. Both the fluorescence components then transmit
25 interference filters 1242 and 1243 and are detected by
the multi-channel photo-multipliers 1012 and 1013,
respectively.

In the embodiment shown in Fig. 13, three

wavelengths are used as the excitation light, while two wavelengths may be used as the excitation light to detect three fluorescence materials. That is, a semiconductor laser having a wavelength of 635 nm and a

5 YAGSHG laser having a wavelength of 532 nm may be used as excitation light sources to detect Cy5 (R), Cy3.5 (R) and Cy3 (R) as fluorescence materials. In this case, since peak values of respective fluorescence spectra are in the vicinity of 670, 570 and 600 nm, the

10 wavelength selection beam splitter 1231 is set to reflect the wavelength of 650 nm or more and transmit the wavelength smaller than 650 nm. Further, the wavelength selection beam splitter 1232 is set to reflect the wavelength of 585 nm or more and transmit

15 the wavelength smaller than 585 nm.

With the detection of fluorescence as described above, by relatively scanning the excitation light and the sample on a slide glass over a desired area of, for example, 20 × 40 mm of the slide glass

20 once, concentration of a plurality of fluorescence materials can be detected as fluorescence intensities.

In the description of the embodiment, the multi-spot array is used as the excitation light by way of example, while even a single spot or excitation

25 light in the form of elongate sheet extending in the direction perpendicular to the scanning direction may be used to attain high-speed detection similarly.

It will be further understood by those

skilled in the art that the foregoing description has been made on embodiments of the invention and that various changes and modifications may be made in the invention without departing from the spirit of the

5   invention and scope of the appended claims.